

## SURVIVIN EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

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**Abstract: Introduction:** Survivin functions as an apoptosis inhibitor and a regulator of cell division. This study aimed to determine the correlation between survivin expression and clinicopathologic parameters of oral squamous cell carcinoma (OSCC) and determine its potential role in the progression/prognosis of this type of tumor.

**Materials and methods:** Immunohistochemical analysis of survivin expression was performed on 45 surgically obtained paraffin-embedded tissue samples of OSCCs. Data on patients' gender, age, tumor grade, site and stage, disease recurrence, metastasis occurrence, and disease-free interval (DFI) were correlated to survivin expression.

**Results:** Survivin immunoreactivity was observed in 77.8% of samples. No significant correlation between survivin expression and age ( $p = 0.087$ ), gender ( $p = 0.334$ ), tumor site ( $p = 0.175$ ), presence of lymph node metastases ( $p = 0.201$ ), or disease recurrence ( $p = 0.451$ ) was found. Survivin expression was observed in well and moderately differentiated tumors and in all clinical stages ( $p = 0.139$ ). Patients with low survivin expression had better survival rates than the group with medium and high survivin expression, i.e., there was a tendency of a shorter DFI in patients with higher expression of survivin ( $p = 0.065$ ).

**Conclusion:** There is a tendency for a shorter disease-free period in patients with higher survivin expression. These data suggest that survivin expression in OSCC may act as an additional prognostic parameter that indicates an increased proliferative tumor potential. To further validate survivin as a prognostic marker in OSCC, a study with a larger sample size along with clinical follow-up data is needed.

**Keywords:** survivin, oral squamous cell carcinoma, prognosis.

### INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a significant public health problem, ranking among the six

most commonly diagnosed malignant tumors worldwide, with a higher prevalence in developing countries (1). Carcinogenesis is a multistage process that may involve not only increased cell proliferation but also decreased cell apoptosis. Survivin is an inhibitor of apoptosis (IAP), which directly inhibits caspase-3 and -7 activity and regulates the cell cycle in the G2/M phase (2).

Survivin is expressed in fetal liver, kidney, and lungs (3), indicating its important role in tissue development (4). However, survivin expression cannot be detected in normal adult tissue, except in thymus tissue, CD34+ stem cells, placenta, basal epithelial cells, hepatocytes, endothelial cells, colon epithelial cells, endometrium, and lymphocytes (5, 6, 7).

Survivin has been recently identified as a promising novel therapeutic target and prognostic marker in different types of cancer (8). Increased survivin expression observed in various precancerous lesions, such as colon epithelial dysplasia and leukoplakia of the oral mucosa, indicates its function in the early stages of carcinogenesis (9, 10).

Survivin expression in tumor cells is most likely independent of the cell cycle, indicating its antiapoptotic role compared to normal cells, where its function in mitosis regulation is dominant. Furthermore, the variable intracellular localization of survivin in tumors (cytoplasmic and nuclear) may serve as an indicator of survivin activity and could potentially act as a prognostic marker for nasopharyngeal carcinoma and astrocytoma (11, 12).

Different studies have found survivin overexpression in poorly differentiated oral squamous cell carcinomas and better survival rates in patients with low expression (13). Except in OSCCs, survivin expression has been found in normal odontogenic epithelium and benign odontogenic lesions. For example, survivin mRNA expression was significantly higher in ameloblastomas than in the epithelium of tooth germs

(14). Various studies have also shown that survivin is expressed in the epithelial cells of pericoronary follicles, follicular cysts, and the basal/suprabasal epithelial layer of keratocystic odontogenic tumors (15, 16). All of these data suggest that survivin participates in the tumorigenesis of the odontogenic epithelium.

The aim of our study was to determine the correlation between survivin expression and clinicopathologic parameters of OSCC and determine its potential role in the progression/prognosis of this type of tumor.

## MATERIAL AND METHODS

This study was conducted at the Clinical Centre of Montenegro from 2012 to 2018 and included 45 patients who required surgical treatment for oral carcinoma localized on the lower lip, tongue, or floor of the mouth. The study was carried out following the principles of the Helsinki Declaration (2002 version) and was approved by the Ethics Committee of the Clinical Center of Montenegro. All patients were followed up for a three-year period, and the time from the beginning of treatment (date of primary surgery) until disease recurrence (disease-free interval, DFI) was used to measure survival rates.

Surgical specimens were formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin for histological analysis. Two independent pathologists, unaware of the participants' clinical status, performed the analysis. Immunohistochemical detection of survivin protein was performed using the DAKO system (Labeled streptavidin-biotin LSAB+ method, DAKO, Denmark) following the manufacturer's instructions. The mouse monoclonal antibody – clone 5B10 (AbD Serotec, Germany) was used for survivin detection.

Positive internal and external staining controls were used, consisting of small salivary glands (presented on several sections) and proximal and distal kidney tubules, respectively.

Survivin expression was assessed in approximately 1000 cells on 10 high-power fields with the highest expression (hot spots) in each specimen. The expression was evaluated semi-quantitatively and categorized based on the percentage of cancer cells stained positive: score 0 indicated no tumor cell reactivity,

score 1 indicated  $\leq 5\%$  positivity, score 2 indicated 5-10% positivity, and score 3 indicated  $\geq 10\%$  tumor cell positivity.

Data on patients' gender, age, tumor grade, site, and stage, as well as disease recurrence, metastasis occurrence, and DFI were correlated with survivin expression using Pearson's chi-squared test. The level of significance was set at 0.05.

## RESULTS

Survivin expression was observed in 77.8% of the cases, with a positive reaction observed in the cytoplasm of tumor cells in all instances. The most frequent category of survivin expression was in samples with less than 5% positive tumor cells (score 1). Low expression of survivin (score 0 and 1) was found in two-thirds of the tumor samples.

Statistical analysis did not reveal statistically significant differences in survivin expression among the groups ( $p = 0.057$ ) (Table 1).

Furthermore, there was no significant correlation between survivin expression and age, gender, tumor site, the presence of lymph node metastases, and disease recurrence (Table 2). Survivin expression was observed in well and moderately differentiated tumors and in all clinical stages, but without statistically significant differences.

However, concerning prognostic significance, it appears that patients with low survivin expression (score 0 and 1) had better survival rates compared to the group with medium and high survivin expression (score 2 and 3). There was a tendency for a shorter disease-free period in patients with higher expression of survivin ( $p = 0.065$ ) (Table 3).

## DISCUSSION

Survivin is known to be predominantly expressed during embryonic development and in fetal tissue, while its expression is weak or absent in normal and differentiated cells (17). However, recent studies have shown significant expression of survivin in mature adult tissues, including basal epithelial cells of the colon, hepatocytes, endothelial cells, endometrium, and lymphocytes (5, 17).

**Table 1.** Immunohistochemical expression of survivin

Variable		N (%)	p value
Survivin expression	score 0	10 (22.2%)	p = 0.057
	score 1: $\leq 5\%$	19 (42.2%)	
	score 2: 5-10%	9 (20.0%)	
	score 3: $\geq 10\%$	7 (15.6%)	

**Table 2.** Statistical analysis of survivin expression and associated clinicopathological findings in OSCC

Variables	N	Survivin expression N (%)	Score 0 N (%)	Score 1 N (%)	Score 2 N (%)	Score 3 N (%)	p-value
Cases	45	35 (77.8)	10 (22.2)	19 (54.2)	9 (25.8)	7 (20.0)	
Age							0.087
< 60	18	16 (88.8)	2 (11.2)	6 (37.5)	7 (43.8)	3 (18.7)	
≥ 60	27	19 (70.3)	8 (29.7)	13 (68.4)	2 (10.5)	4 (21.1)	
Gender							0.334
Male	36	30 (83.3)	6 (16.7)	16 (53.3)	9 (30.0)	5 (16.7)	
Female	9	5 (55.5)	4 (44.5)	3 (60.0)	0 (0.0)	2 (40.0)	
Grading							0.139
G1	24	20 (83.3)	4 (16.7)	9 (45.0)	7 (35.0)	4 (20.0)	
G2	21	16 (76.0)	5 (24.0)	10 (62.0)	3 (19.0)	3 (19.0)	
Staging							0.139
I	14	12 (86.0)	2 (14.0)	6 (50.0)	2 (17.0)	4 (33.0)	
II	10	8 (80.0)	2 (20.0)	4 (50.0)	2 (25.0)	2 (25.0)	
III	7	6 (86.0)	1 (14.0)	4 (67.0)	1 (16.5)	1 (16.5)	
IV	14	10 (71.0)	4 (29.0)	4 (40.0)	4 (40.0)	2 (20.0)	
Tumor site							0.175
Lip	19	11 (58.0)	8 (42.0)	5 (46.0)	3 (27.0)	3 (27.0)	
Tongue/ floor of mouth and tongue	26	24 (92.0)	2 (8.0)	14 (58.0)	6 (25.0)	4 (17.0)	
Metastasis (node)							0.201
Positive (N+)	18	12 (67.0)	6 (33.0)	6 (50.0)	5 (42.0)	1 (8.0)	
Disease recurrence	5	4 (80.0)	1 (20.0)	2 (50.0)	0 (0.0)	2 (50.0)	0.451

**Table 3.** Survivin expression and DFI (disease free interval)

	Survivin expression (0 i 1)	Survivin expression (2 i 3)	p-value
DFI (mean value - months)	18	12	p = 0,065

The subcellular localization of survivin includes the nucleus, cytoplasm, and mitochondria (18). It has been suggested that cytoplasmic survivin plays a crucial role in the survival of tumor cells by acting as an inhibitor of apoptosis, while nuclear survivin contributes to cell proliferation and helps maintain the integrity of the mitotic spindle. Consequently, higher expression of nuclear survivin may indicate accelerated mitotic processes, which can have a negative prognostic value in certain types of tumors (19).

In our study, we observed that survivin expression was exclusively cytoplasmic in all examined cases. The majority of the samples (2/3) showed low survivin expression, with little or no reactivity of tumor cells (less than 5% positive tumor cells). This is consistent with the characteristic expression pattern observed in many normal, differentiated human cells (17), as per the DAKO protocol used in our investigation. Therefore, we set the cut-off value for survivin overexpression in our study group at > 5%. Increased cytoplasmic survivin expression was detected in only a third of our patients (35.6%). However, it is essential to consider

that half of the sample consisted of patients with lip cancer, which is typically well-differentiated and has a favorable prognosis due to early diagnosis. This may explain the lower expression of survivin observed in our cohort.

In contrast, some other malignancies, such as non-small cell lung cancer, pancreatic and colon cancers, soft tissue sarcomas, melanomas, and neuroblastoma, have demonstrated increased cytoplasmic expression of survivin (4, 20-26). The expression of cytoplasmic survivin has been identified as a negative prognostic factor in malignant tumors of the salivary glands, colon, and squamous cell carcinomas of the oral cavity (13, 26, 27). Our study also indicated a tendency towards a shorter disease-free period in patients with higher survivin expression. However, the possibility of different results on a larger study sample cannot be excluded.

Engels et al. investigated the relationship between cytoplasmic and nuclear survivin and its impact on prognosis in patients with OSCC (11). They observed that a change in the ratio of nuclear to cytoplasmic

survivin expression was a positive prognostic factor concerning the duration from the end of treatment to disease recurrence (relapse-free survival). In vitro experiments revealed that the intracellular localization of survivin is regulated by active transport from the nucleus to the cytoplasm, mediated by the specific receptor Crml and the corresponding sequence of amino acids within the protein known as NES (nuclear export signal). This mechanism appears to play a crucial role in the cytoprotective function of survivin, as exposure of tumor cells to cisplatin or radiation leads to the transport of survivin from the nucleus to the cytoplasm, reducing the sensitivity of cells to chemotherapy and radiotherapy (11).

## CONCLUSION

In conclusion, our study revealed a tendency towards a shorter disease-free period in patients with higher survivin expression in oral squamous cell carcinoma (OSCC). This suggests that survivin expression may serve as an additional prognostic parameter, indicating an increased proliferative tumor potential. However, to further validate survivin as a prognostic

marker in OSCC, larger studies with a greater sample size and comprehensive clinical follow-up data are required. Such investigations would contribute to a better understanding of the role of survivin in OSCC progression and aid in developing targeted therapeutic strategies for improved patient outcomes.

## Abbreviations

**OSCC** — oral squamous cell carcinoma

**DFI** — disease free interval

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## Sažetak

# EKSPRESIJA SURVIVINA U ORALNOM SKVAMOCELULARNOM KARCINOMU

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**Uvod:** Survivin je inhibitor apoptoze i regulator ćelijske deobe. Cilj ovog istraživanja je bio da se utvrdi korelacija između ekspresije survivina i kliničko-patoloških parametara oralnog skvamocelularnog karcinoma (OSCC) kao i njegova potencijalna uloga u progresiji/proгноzi ove vrste tumora. **Materijal i metode:** Imunohistohemijska analiza ekspresije survivina je sprovedena na 45 hirurški odstranjenih i parafinski ukalupljenih uzoraka oralnih skvamocelularnih karcinoma. Podaci o polu i starosti pacijenata, gradusu tumora, lokalizaciji i stadijumu, recidivu bolesti, pojavi metastaza i intervalu bez bolesti (DFI) su upoređeni sa ekspresijom survivina. **Rezultati:** Imunoreaktivnost na survivin je utvrđena u 77.8% uzoraka. Nije utvrđena značajna povezanost između ekspresije survivina i starosti ( $p = 0.087$ ), pola ( $p = 0.334$ ), lokalizacije tumora ( $p = 0.175$ ) ili prisustva regionalnih metastaza ( $p = 0.201$ ) i recidiva bolesti ( $p = 0.451$ ). Ekspresija

survivina je bila prisutna u dobro i umereno diferentovanim tumorima i u svim kliničkim stadijumima ( $p = 0.139$ ). Pacijenti sa niskom ekspresijom survivina su imali bolje stope preživljavanja u odnosu na pacijente sa srednjom i visokom ekspresijom survivina tj. utvrđena je tendencija prisustva kraćeg DFI kod pacijenata sa višim nivoom ekspresije survivina ( $p = 0.065$ ). **Zaključak:** Postoji tendencija kraćeg perioda bez bolesti kod pacijenata sa većom ekspresijom survivina. Ovi podaci upućuju da bi ekspresija survivina u oralnom skvamocelularnom karcinomu mogla biti dodatni prognostički parametar koji ukazuje na povećan proliferativni potencijal tumora. Da bi se survivin potvrdio kao prognostički parametar u oralnom skvamocelularnom karcinomu, potrebna je studija na većem uzorku pacijenata uz njihovo kliničko praćenje.

**Ključne reči:** survivin, oralni skvamocelularni karcinom, prognoza.



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